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In-Vitro Hemolysis and Its Effect on Peripheral Blood Counts

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Abstract

Objective: To evaluate the degree of interference caused by hemolyzed samples on Complete Blood Counts (CBC) parameters analyzed by Sysmex XN-1000.

Methodology: This cross-sectional study conducted at Chughtai Institute of Pathology from August, 2023 to August, 2024 after securing approval from the Research and Ethical committee. Peripheral venous blood samples of 100 patients were collected in potassium ethylenediaminetetraacetic acid (K2-EDTA) vials and were divided into three equal aliquots. The Aliquot-1 was subjected to no interference whereas Aliquot 2 and 3 were subjected to 5 and 10 times of interference by passing through a narrow caliber needle to create mechanical hemolysis. All these aliquots were then run on Sysmex XN-1000 automated analyzer for the CBC test. Three study groups were made based on the amount of interference and Hemolysis Index (HI).

Results: As compared to non-hemolysed samples, the interference at different levels affects various haematological parameters to varying extents. Hematocrit and platelet counts demonstrate the most substantial variability, whereas WBC counts and MCHC are relatively robust against interference.

Conclusion: This study demonstrates that the in-vitro hemolysis can have direct negative impact on the results of the CBC parameters. Thus, this preanalytical error must not be overlooked in order to safeguard patient's best interests.

Key words: Hemolysis, interference, preanalytical variability.

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Introduction

The preanalytical phase of sample analysis involves specimen collection, handling, delivery and storage. This phase of the total analytical process involves the major number of errors.[1] Around 60% of sample rejections by a laboratory are a result of hemolyzed samples, thus delaying patient care.[2] Hemolysis can occur both in-vitro and in-vivo. The in-vivo hemolysis occurs due to the patient's own pathological processes (acquired/inherited causes of hemolysis).[3]

In-vitro hemolysis or hemolyzed samples are common forms of preanalytical errors which can lead to erroneous results in complete blood count analysis.[4] This type of hemolysis occurs when venous sampling of a patient is performed using Intravenous (IV) catheters, incorrect handling of the sample, vigorous shaking, unsuitable temperature or difficult sampling. During the passage the Red Blood Cells (RBCs) membranes become fragile and consequently rupture leading to spilling of its contents like hemoglobin, potassium, magnesium, phosphate bilirubin, Lactate Dehydrogenase (LDH), Aspartate aminotransferase (AST) etc.[5]

When such samples are analysed by hematology or even chemistry department analyzers, these spilled contents produce interference leading to unreliable results. The degree of interference depends on the analyte/parameter being measured.[6] These samples are a source of delay in patient care due to rejection and redrawing of sample, also leading to increased financial burden.[7] It is very difficult to assess whether the hemolysis was caused by ex-vivo or in-vivo phenomena.

It is important for a laboratory to conduct research and ascertain to what degree an interference can occur while measuring certain parameters. A faulty diagnosis based on such samples can lead to a devastating effect on the patient´s management.[8] Not many studies have been conducted to measure the influence of hemolyzed samples on Complete Blood Count parameters involving automated analyzers which analyze by impedance and flow cytometry methods. So, the aim of this study was to evaluate the degree of interference caused by hemolyzed samples on CBC parameters analyzed by an automated hematology analyzer using impedance and flow cytometry methods.[9]

Methodology

It was a cross-sectional study conducted at Chughtai

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Institute of Pathology from August, 2023 to August, 2024 after securing approval from the Research and Ethical committee. A total of 100 both male and female volunteers were included in the study after taking informed consent. 3 mL of peripheral venous blood samples of these volunteers were collected in potassium ethylenediaminetetraacetic acid (K2-EDTA) vials and gently mixed. Any traumatic venous puncture was excluded from the study.

These samples were divided into three equal aliquots of 1 mL. The Aliquot-1 was left as such and the Aliquot-2 and Aliquote-3 of each sample were subjected to mechanical hemolysis by the Demeski method.[10] Whole blood in these samples were passed through a narrow-gauge needle (24G) to elicit variable amounts of hemolysis. Sample in Aliquot-2 was passed 5 times whereas the one in Aliquot-3 was passed 10 times through the narrow caliber needle.

All these aliquots were then run on Sysmex XN-1000 automated analyzer for the CBC test. The analyzer uses the principles of impedance and flow cytometry to determine the counts. The hematological parameters included in our study are as follows: Hemoglobin (Hb), Hematocrit (HCT), White Blood Cells count (WBC), Platelet count (PLT), RBC count, mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Afterwards, these samples were centrifuged to separate plasma and were sent to the Chemistry Department for measurement of plasma free hemoglobin (fHB) and calculation of Hemolysis Index (HI) by Alinity Ci.[11] This instrument uses spectrophotometric method to perform HI assay.[12] Three study groups were made based on the amount of interference and HI: Group I with no interference and minimal hemolysis, Group II with 5 times interference and moderate amount of hemolysis and Group III with 10 times interference and marked degree of haemolysis.

The statistical analysis of differences between the samples was assessed by paired Student's t-test. Bland & Altman plots and limits-of-agreement analysis were used to compare results on aliquots. The plot differences were finally reported as percentage variations (mean and 95% confidence of interval, 95% CI) and compared with the analytical quality specifications for desirable bias.

Results

The statistical analysis of various hematological parameters and their respective mean differences under different interference conditions indicates varying degrees of bias and limits of agreement. In hemolysis, comparisons between no interference and 5 times interference, as well as between 5 times and 10 times interference, demonstrate a consistent mean difference of -0.81 with the 95% confidence intervals remaining negative, suggesting systematic underestimation. However, the agreement limits vary slightly, reflecting minor variations in measurement dispersion.

For hemoglobin, the mean differences are close to zero but predominantly negative, with 95% confidence intervals indicating potential underestimation across comparisons. The limits of agreement remain narrow, particularly for 5 times vs. 10 times interference, where the dispersion is minimal compared to other comparisons. This consistency suggests reliable but slightly biased measurements across interference conditions. In hematocrit, there is substantial variability, particularly between no interference and 5 times interference, where the limits of agreement are extremely wide. The mean differences for this parameter range from being slightly negative to positive, depending on the comparison, reflecting inconsistent bias under different interference levels.

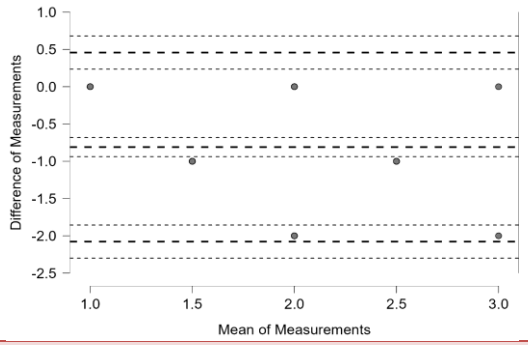
White blood cell (WBC) counts show small positive mean differences between no interference and interference conditions, with tighter agreement limits compared to other parameters. This suggests minimal bias and good reliability in measurements. However, for 5 times vs. 10 times interference, the mean difference is close to zero with overlapping confidence intervals, indicating negligible change under these conditions.

Platelet counts exhibit a consistent negative mean difference across comparisons, with wider limits of agreement for higher interference levels. This reflects a systematic underestimation that worsens as interference increases. Similarly, red blood cell (RBC) counts show minimal mean differences close to zero under lower interference conditions but demonstrate larger discrepancies, with broader limits at higher interference levels.

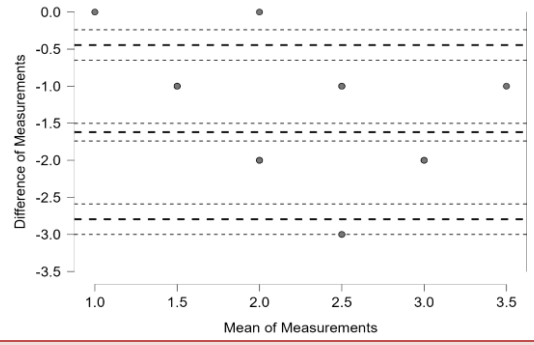
Table I: Bland–Altman Biase and limits for different hematological parameters on different interference (no, at 5 times and 10 times)

	Bias & Limits	Point Value	Lower 95% CI	Upper 95% CI
Hemolysis[No interference vs. 5 times interference]	Mean difference + 1.96 SD	0.457	0.235	0.68
	Mean difference	-0.81	-0.938	-0.682
	Mean difference - 1.96 SD	-2.077	-2.3	-1.855
Hemolysis [No interference vs. 10 times interference]	Mean difference + 1.96 SD	-0.445	-0.651	-0.239
	Mean difference	-1.62	-1.739	-1.501
	Mean difference - 1.96 SD	-2.795	-3.001	-2.589
Hemolysis [5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	0.457	0.235	0.68
	Mean difference	-0.81	-0.938	-0.682
	Mean difference - 1.96 SD	-2.077	-2.3	-1.855
Hemoglobin (g/dL) [No interference vs. 5 times interference]	Mean difference + 1.96 SD	1.024	0.815	1.232
	Mean difference	-0.165	-0.285	-0.045
	Mean difference - 1.96 SD	-1.354	-1.562	-1.145
Hemoglobin (g/dL) [No interference vs. 10 times interference]	Mean difference + 1.96 SD	1.022	0.812	1.231
	Mean difference	-0.173	-0.294	-0.052
	Mean difference - 1.96 SD	-1.368	-1.577	-1.158
Hemoglobin (g/dL) [5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	0.752	0.619	0.886
	Mean difference	-0.008	-0.085	0.069
	Mean difference - 1.96 SD	-0.768	-0.902	-0.635
Hematocrit (%)([No interference vs. 5 times interference]	Mean difference + 1.96 SD	680.908	554.894	806.922
	Mean difference	-37.755	-110.51	34.999
	Mean difference - 1.96 SD	-756.419	-882.433	-630.404
Hematocrit (%) [No interference vs. 10 times interference]	Mean difference + 1.96 SD	4.274	3.243	5.305
	Mean difference	-1.606	-2.201	-1.011
	Mean difference - 1.96 SD	-7.486	-8.517	-6.455
Hematocrit (%) [5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	754.614	628.635	880.593
	Mean difference	36.149	-36.585	108.884
	Mean difference - 1.96 SD	-682.315	-808.295	-556.336
WBC count (x 10^9/L) [No interference vs. 5 times interference]	Mean difference + 1.96 SD	3.281	2.815	3.747
	Mean difference	0.624	0.355	0.893
	Mean difference - 1.96 SD	-2.033	-2.498	-1.567
WBC count (x 10^9/L)[No interference vs. 10 times interference]	Mean difference + 1.96 SD	3.845	3.297	4.392
	Mean difference	0.722	0.406	1.038
	Mean difference - 1.96 SD	-2.401	-2.949	-1.854
WBC count (x 10^9/L)[5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	2.113	1.759	2.466
	Mean difference	0.098	-0.106	0.302
	Mean difference - 1.96 SD	-1.917	-2.271	-1.564
Platelet count (x 10^9/L)[No interference vs. 5 times interference]	Mean difference + 1.96 SD	62.227	47.276	77.178
	Mean difference	-23.04	-31.672	-14.408
	Mean difference - 1.96 SD	-108.307	-123.258	-93.356
Platelet count (x 10^9/L) [No interference vs. 10 times interference]	Mean difference + 1.96 SD	76.189	57.976	94.401

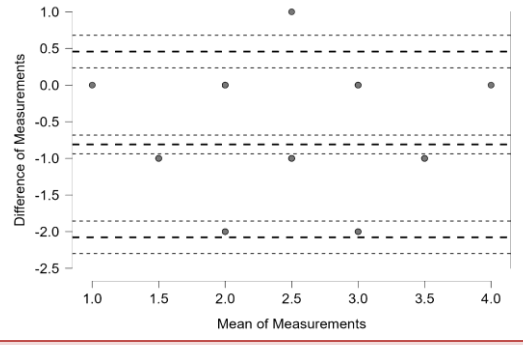
	Mean difference	-27.678	-38.193	-17.163
	Mean difference - 1.96 SD	-131.545	-149.757	-113.332
Platelet count (x 10^9/L)[5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	64.087	52.037	76.138
	Mean difference	-4.638	-11.595	2.319
	Mean difference - 1.96 SD	-73.363	-85.414	-61.313
RBC count (x 10^12/L)[No interference vs. 5 times interference]	Mean difference + 1.96 SD	0.429	0.358	0.5
	Mean difference	0.025	-0.016	0.066
	Mean difference - 1.96 SD	-0.379	-0.449	-0.308
RBC count (x 10^12/L)[No interference vs. 10 times interference]	Mean difference + 1.96 SD	16.758	13.666	19.849
	Mean difference	-0.873	-2.657	0.912
	Mean difference - 1.96 SD	-18.503	-21.594	-15.411
RBC count (x 10^12/L)[5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	16.72	13.631	19.809
	Mean difference	-0.898	-2.681	0.886
	Mean difference - 1.96 SD	-18.515	-21.605	-15.426
MCV (fL)[No interference vs. 5 times interference]	Mean difference + 1.96 SD	10.67	8.034	13.306
	Mean difference	-4.363	-5.885	-2.841
	Mean difference - 1.96 SD	-19.396	-22.032	-16.76
MCV (fL)[No interference vs. 10 times interference]	Mean difference + 1.96 SD	11.813	8.837	14.789
	Mean difference	-5.158	-6.876	-3.44
	Mean difference - 1.96 SD	-22.129	-25.105	-19.153
MCV (fL)[5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	6.64	5.336	7.943
	Mean difference	-0.795	-1.548	-0.042
	Mean difference - 1.96 SD	-8.23	-9.533	-6.926
MCH (pg)[No interference vs. 5 times interference]	Mean difference + 1.96 SD	2.581	2.025	3.136
	Mean difference	-0.587	-0.908	-0.266
	Mean difference - 1.96 SD	-3.755	-4.31	-3.199
MCH (pg)[No interference vs. 10 times interference]	Mean difference + 1.96 SD	2.559	1.97	3.149
	Mean difference	-0.803	-1.143	-0.462
	Mean difference - 1.96 SD	-4.165	-4.754	-3.575
MCH (pg)[5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	2.803	2.274	3.333
	Mean difference	-0.216	-0.521	0.09
	Mean difference - 1.96 SD	-3.235	-3.764	-2.705
MCHC (%)[No interference vs. 5 times interference]	Mean difference + 1.96 SD	4.562	3.885	5.239
	Mean difference	0.699	0.308	1.09
	Mean difference - 1.96 SD	-3.164	-3.842	-2.487
MCHC (%)[No interference vs. 10 times interference]	Mean difference + 1.96 SD	5.175	4.421	5.929
	Mean difference	0.875	0.44	1.31
	Mean difference - 1.96 SD	-3.425	-4.179	-2.671
MCHC (%)[5 times interference vs. 10 times interference]	Mean difference + 1.96 SD	2.182	1.83	2.533
	Mean difference	0.176	-0.027	0.379
	Mean difference - 1.96 SD	-1.83	-2.181	-1.478



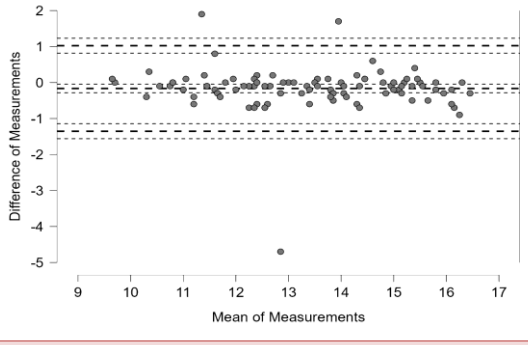
Hemolysis[No interference]



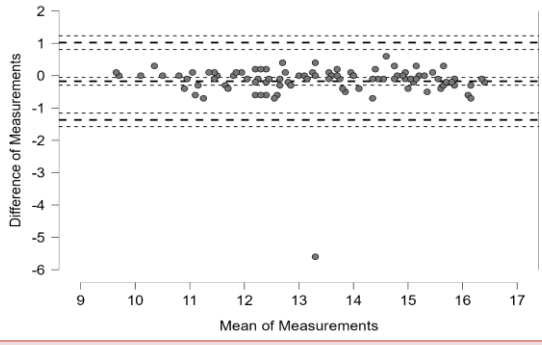
Hemolysis [No interference vs. 10 times interference]



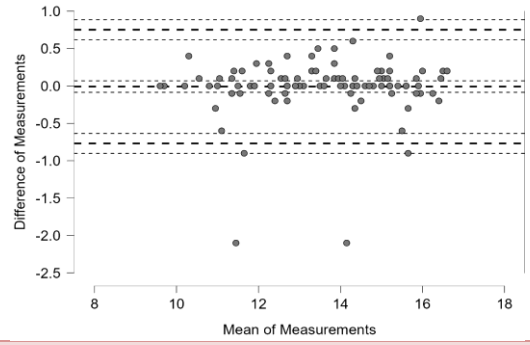
Hemolysis [5 times interference vs. 10 times interference]



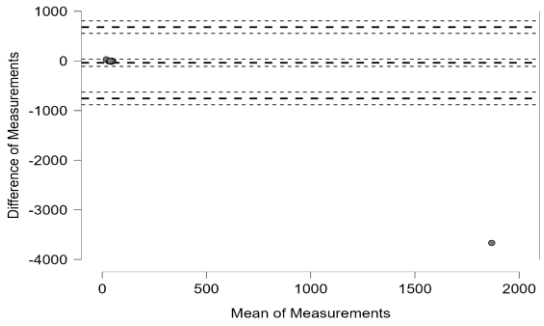
Hemoglobin (g/dL) [No interference vs. 5 times interference]



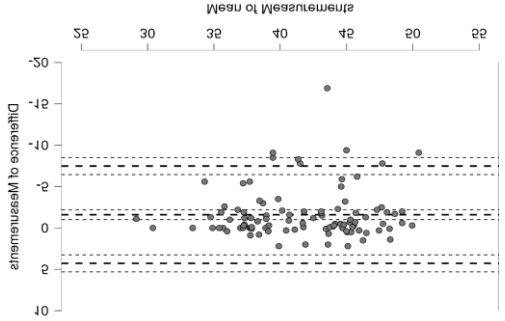
Hemoglobin (g/dL) [No interference vs. 10 times interference]



Hemoglobin (g/dL) [5 times interference vs. 10 times interference]

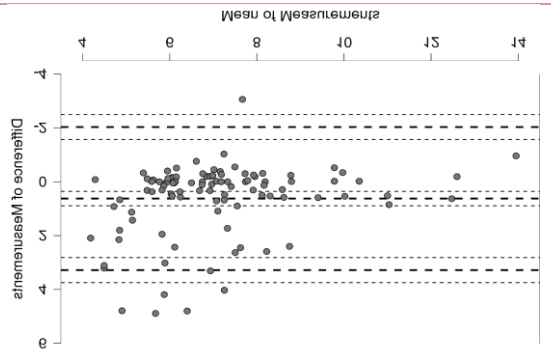


Hematocrit (%)([No interference vs. 5 times interference]

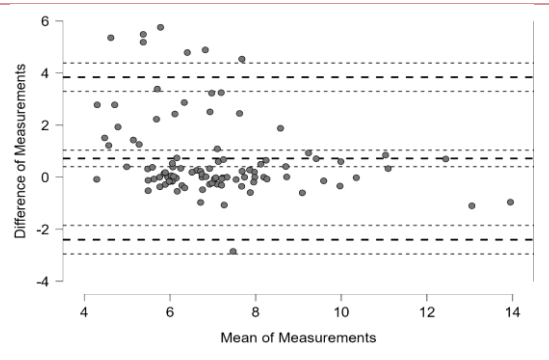


Hematocrit (%) [No interference vs. 10 times interference]

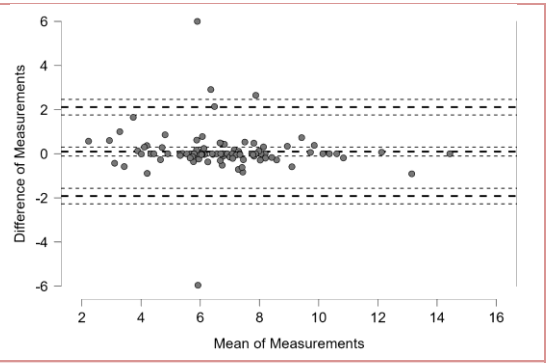
Hematocrit (%) [5 times interference vs. 10 times interference]



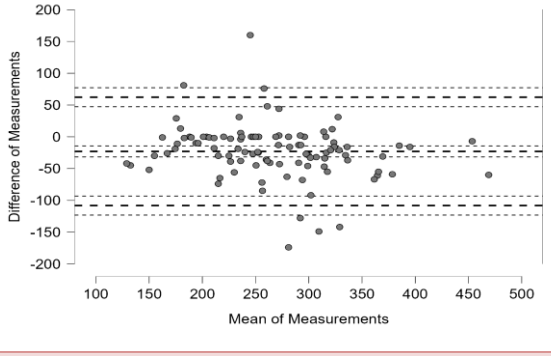
WBC count (x 10^9/L) [No interference vs. 5 times interference]



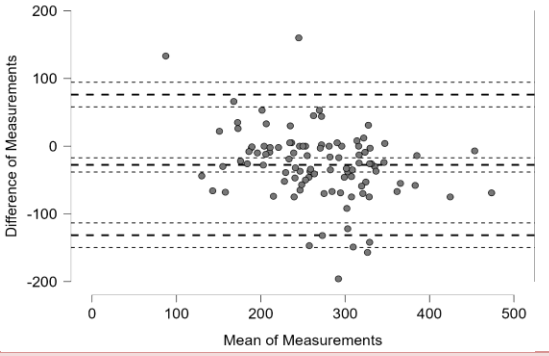
WBC count (x 10^9/L)[No interference vs. 10 times interference]



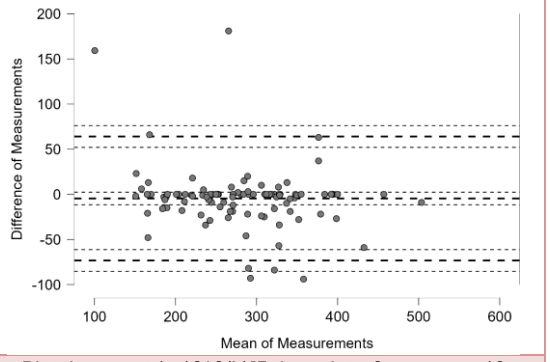
WBC count (x 10^9/L)[5 times interference vs. 10 times interference]



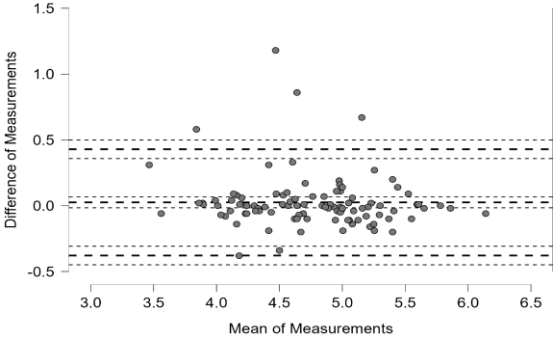
Platelet count (x 10^9/L)[No interference vs. 5 times interference]



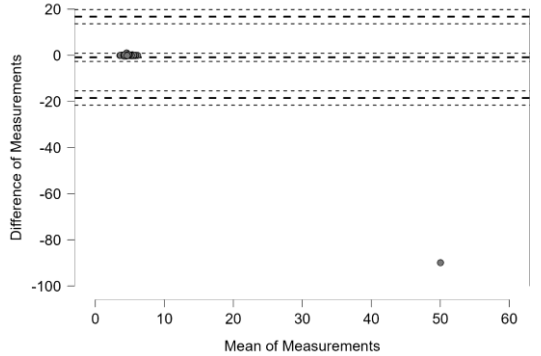
Platelet count (x 10^9/L) [No interference vs. 10 times interference]



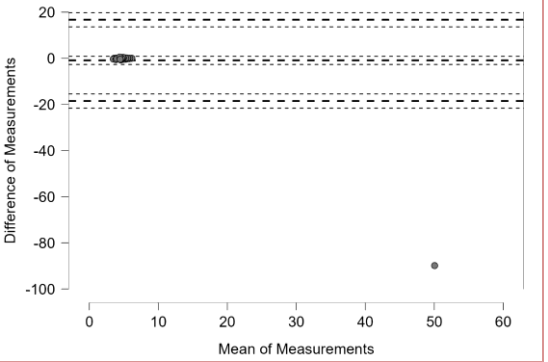
Platelet count (x 10^9/L)[5 times interference vs. 10 times interference]



RBC count (x 10^12/L)[No interference vs. 5 times interference]



RBC count (x 10^12/L)[No interference vs. 10 times interference]



RBC count (x 10^12/L)[5 times interference vs. 10 times interference]

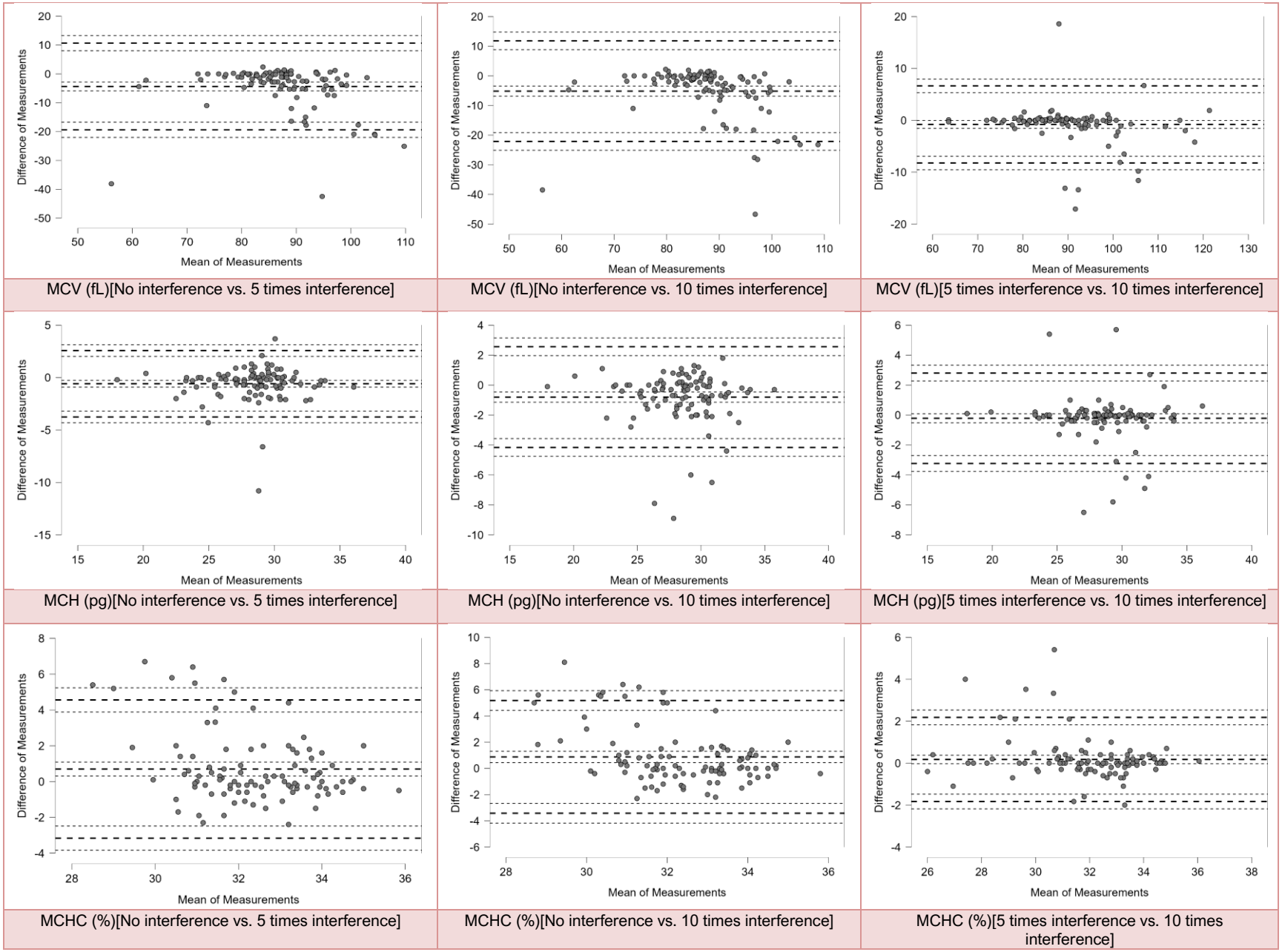


Figure 1. Bland–Altman plot for different hematological parameters on different interference (no, at 5 times and 10 times)

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) display a systematic decrease in mean values with increasing interference, supported by negative mean differences and overlapping confidence intervals. The limits of agreement, while moderately broad, suggest variability that may be attributable to interference effects. Conversely, mean corpuscular hemoglobin conditions, with narrow confidence intervals and agreement limits, indicating consistent measurements with minimal bias.

Discussion

The results of our study show that hemolysis poses a threat to the actual results of CBC thereby it is important to negate this pre-analytical error before proceeding further. In a study conducted by Lippi et al they observed low RBC count and HCT, whereas increased MCHC and platelet count in samples with greater hemolysis.[1] Whereas in our study also MCHC increased.

As observed by Lippi G et al, there was gradual decrease in RBCs count and hematocrit as hemolysis increased which can be explained by the fact that RBCs ruptured causing increase in both LDH and HI.[13] In our study too red blood cell (RBC) counts show minimal mean differences close to zero under lower interference conditions but demonstrate larger discrepancies, with broader limits at higher interference levels.

De Jonge et al observed in their study alteration in platelet count. [14] The possible explanation for this is that as hemolysis increases, it means that the sample will have a greater number of RBC fragments. These RBC fragments and debris will be counted as platelets by automated hematological analysers.[15] Whereas, contrary to that in our study platelet counts exhibit a consistent negative mean difference across comparisons, with wider limits of agreement for higher interference levels.

Conclusion

Interference at different levels affects various hematological parameters to varying extents. Hematocrit and platelet counts demonstrate the most substantial variability, whereas WBC counts and MCHC are relatively robust against interference. These findings highlight the importance of accounting for potential biases and variability in laboratory measurements under interference conditions to ensure accuracy and reliability.

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